

Decreased Dendritic Spine Density on Prefrontal Cortical Pyramidal Neurons in Schizophrenia

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Background: The pathophysiological characteristics of schizophrenia appear to involve altered synaptic connectivity in the dorsolateral prefrontal cortex. Given the central role that layer 3 pyramidal neurons play in corticocortical and thalamocortical connectivity, we hypothesized that the excitatory inputs to these neurons are altered in subjects with schizophrenia.

Methods: To test this hypothesis, we determined the density of dendritic spines, markers of excitatory inputs, on the basilar dendrites of Golgi-impregnated pyramidal neurons in the superficial and deep portions of layer 3 in the dorsolateral prefrontal cortex (area 46) and in layer 3 of the primary visual cortex (area 17) of 15 schizophrenic subjects, 15 normal control subjects, and 15 nonschizophrenic subjects with a psychiatric illness (referred to as psychiatric subjects).

Results: There was a significant effect of diagnosis on spine density only for deep layer 3 pyramidal neurons

in area 46 ($P = .006$). In the schizophrenic subjects, spine density on these neurons was decreased by 23% and 16% compared with the normal control ($P = .004$) and psychiatric ($P = .08$) subjects, respectively. In contrast, spine density on neurons in superficial layer 3 in area 46 ($P = .09$) or in area 17 ($P = .08$) did not significantly differ across the 3 subject groups. Furthermore, spine density on deep layer 3 neurons in area 46 did not significantly ($P = .81$) differ between psychiatric subjects treated with antipsychotic agents and normal controls.

Conclusion: This region- and disease-specific decrease in dendritic spine density on dorsolateral prefrontal cortex layer 3 pyramidal cells is consistent with the hypothesis that the number of cortical and/or thalamic excitatory inputs to these neurons is altered in subjects with schizophrenia.

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THE DORSOLATERAL prefrontal cortex (DLPFC) appears to be a critical site of dysfunction in subjects with schizophrenia. For example, schizophrenic subjects perform poorly on cognitive tasks that are subserved by DLPFC circuitry,^{1,2} and this performance deficit is correlated with diminished activation of the DLPFC.^{2,3}

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The results of structural imaging studies⁴⁻⁸ suggest that DLPFC dysfunction in subjects with schizophrenia may be related to a decreased volume of this brain region. Findings of increased cell packing density,⁹⁻¹¹ without a change in neuronal number,^{12,13} suggest that this volume reduction may be due to a decreased amount of DLPFC neuropil in subjects with schizophrenia.¹⁴ Because the axon terminals and dendritic spines of the neuropil represent the major anatomical substrate for synapses, these findings suggest the presence of abnormalities in DLPFC connectivity in subjects with schizophrenia.

Consistent with this interpretation, magnetic resonance spectroscopic stud-

ies¹⁵⁻¹⁸ have found decreased levels of *N*-acetylaspartate, a putative marker of neuronal and/or axonal integrity, in the DLPFC of schizophrenic subjects. Reports¹⁹⁻²¹ of decreased phosphomonoesters, increased phosphodiesterases, or both in the DLPFC of schizophrenic subjects have also been interpreted to reflect an increased breakdown of membrane phospholipids and, consequently, a decreased number of synapses.²² Finally, levels of synaptophysin, a presynaptic terminal protein, are decreased in the DLPFC of schizophrenic subjects.²³⁻²⁶ Although other interpretations are possible, each of these findings supports the hypothesis that DLPFC synaptic number is diminished in subjects with schizophrenia.

Understanding the pathophysiological significance of such alterations requires knowledge of the types of synapses that are affected and of their postsynaptic targets. Several findings suggest that schizophrenia may be associated with abnormalities in the excitatory inputs to layer 3 pyramidal neurons in the DLPFC. For example, the basilar dendritic spines of pyramidal cells in deep layer 3 are likely targets of projections from the mediodorsal thalamic nucleus,^{27,28} and

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SUBJECTS AND METHODS

SUBJECT CHARACTERISTICS

Specimens from 45 human brains were obtained during autopsies conducted at the Allegheny County Coroner's Office, Pittsburgh, Pa (**Table 1**). Informed consent for brain donation was obtained from the next of kin. Neuropathological abnormalities were detected in 6 subjects. Subject 517 had a vascular malformation and hemorrhage confined to the right temporal lobe, and subject 622 had an acute infarction limited to the distribution of the right middle cerebral artery. However, the cortical regions of interest for the present study were not affected in either subject. In 4 subjects (subjects 532, 564, 609, and 632), thioflavine S staining revealed a few senile plaques without any neurofibrillary tangles. The density of plaques was insufficient to meet the diagnostic criteria for Alzheimer disease,³⁷ and there was no history of dementia in any subject.

Fifteen subjects with a diagnosis of schizophrenia or schizoaffective disorder were compared with 15 normal control subjects and 15 nonschizophrenic subjects with a psychiatric illness (referred to as psychiatric subjects) (Table 1). For each subject, an independent committee of experienced clinicians made consensus *DSM-III-R*³⁸ diagnoses using information obtained from clinical records and structured interviews conducted with surviving relatives of the subject.²³ One of the normal controls (subject 370) was later found to have a diagnosis of alcohol abuse, current at the time of death. Thirteen of the subjects in the psychiatric comparison group had a mood disorder, and 2 had other psychotic disorders. All of the schizophrenic subjects had a history of treatment with antipsychotic agents, and 9 of the psychiatric subjects had been treated with these medications. All procedures were approved by the Institutional Review Board for Biomedical Research at the University of Pittsburgh, Pittsburgh, Pa.

Subject groups did not differ significantly in sex, race, age, postmortem interval (PMI), tissue fixation time, or incidence of out-of-hospital deaths (**Table 2**). In addition, the schizophrenic and psychiatric subjects did not differ on the incidence of alcohol or other substance use disorders, mean age at onset of illness, or mean duration of illness. However, the number of deaths by suicide was significantly higher in the psychiatric subjects (Table 2).

PREPARATION OF TISSUE

From the left hemisphere of each brain, tissue blocks were cut from standardized locations in the DLPFC and primary visual cortex, immersed in 4% paraformaldehyde for longer than 4 weeks, and then processed by a previously described modification³⁹ of the rapid Golgi procedure.⁴⁰ Sections were cut at 90 μ m and mounted onto coded slides so that investigators were not aware of subject number or diagnosis.

Area 46 of the DLPFC and area 17, primary visual cortex, were examined in this study. Area 17 was not available for 2 schizophrenic subjects (subjects 410 and 622) and 1 psychiatric subject (subject 637). Nissl-stained sections from tissue blocks immediately adjacent to those used for the Golgi procedure were examined to confirm that the Golgi material contained the cytoarchitectural features of areas 46 or 17.^{9,41} The Nissl-stained sections were also used to determine the borders of layer 3 as a percentage of total cortical thickness. In area 46, the layer 2 to 3 border was located on average (\pm SD) 18.2% (\pm 3.4%) and 20.8% (\pm 2.8%) of the distance from the pial surface to the white matter for the schizophrenic and control subjects, respectively, and the layer 3 to 4 border was located at 53.6% (\pm 3.1%) and 56.8% (\pm 1.2%) of the total cortical thickness in these 2 subject groups, respectively. These findings are consistent with previous reports^{11,42} that the relative thicknesses of cortical layers are similar in schizophrenic and control subjects. Consequently, Golgi-impregnated neurons located between 20%

the number of neurons in this nucleus appears to be decreased in subjects with schizophrenia.²⁹⁻³¹ In addition, excitatory inputs to layer 3 pyramidal cells decline substantially in number during late adolescence,³²⁻³⁴ the typical age when the clinical features of schizophrenia become manifest.

Consequently, the purpose of this study was to test the hypothesis that schizophrenia is associated with a diminished complement of excitatory synapses onto DLPFC layer 3 pyramidal neurons. Because dendritic spines are the principal site of excitatory inputs to pyramidal neurons,³⁵ and reflect the number of these synapses,^{35,36} we quantified dendritic spine density on the basilar dendrites of layer 3 pyramidal neurons in schizophrenic subjects and 2 groups of comparison subjects.

RESULTS

SPINE DENSITY ON LAYER 3 PYRAMIDAL NEURONS

The Golgi impregnation procedure clearly filled the basilar dendritic shafts and spines of layer 3 pyramidal neu-

rons. As shown in **Figure 2**, differences in spine density among neurons and across brains were sometimes quite evident.

In DLPFC area 46 (**Table 3**), the mean spine density on the basilar dendrites of pyramidal neurons in superficial layer 3 of the schizophrenic subjects was 15% lower than that of the normal controls and 13% lower than that of the psychiatric subjects (**Figure 3, A**). However, these differences did not achieve statistical significance ($F_{2,37} = 2.52, P = .09$).

In contrast, the spine density on the basilar dendrites of pyramidal neurons in deep layer 3 of area 46 did significantly differ ($F_{2,37} = 6.01, P = .006$) among the 3 subject groups (Table 3). There was also a main effect for age ($F_{1,37} = 8.49, P = .006$) on spine density, but reanalysis failed to reveal a significant age-by-diagnosis interaction ($F_{2,36} = 1.87, P = .17$). In addition, there was no effect of race, sex, PMI, or tissue fixation time ($F_{1,37} < 0.86, P > .47$) on spine density.

As shown in Figure 3, B, the mean spine density on pyramidal neurons in deep layer 3 was 23% lower (95% confidence interval, -42.3% to -6.7%) in the schizophrenic subjects than in the normal controls, a differ-

and 55% of the distance from the pial surface to the white matter in area 46 were considered to be located in layer 3. In Golgi-processed sections containing area 17, a dark band demarcated layer 4.⁴⁰ Therefore, sampled neurons were located superficially to this band or within 20% to 45% of the distance from the pial surface to the white matter.

NEURON RECONSTRUCTIONS

Golgi-impregnated pyramidal neurons were readily identified by their characteristic triangular somal shape, apical dendrite extending toward the pial surface, and numerous dendritic spines. The following criteria were used to select pyramidal neurons for reconstruction: (1) location of the cell soma in layer 3 and within the middle of the thickness (*z*-axis) of the section; (2) full impregnation of the neuron; (3) soma or dendrites not obscured by overlying opaque artifacts larger than 5 μm ; (4) no morphologic changes attributable to PMI⁴³; and (5) presence of at least 3 primary basilar dendritic shafts, each of which branched at least once. For each subject, 15 neurons were randomly sampled in each of 3 locations: (1) the superficial half of layer 3 (20%-37% of the total cortical depth) in area 46, (2) the deep half of layer 3 (38%-55% of the total cortical depth) in area 46, and (3) layer 3 of area 17. The adequacy of these sampling procedures for detecting differences in spine density has been previously demonstrated.^{32,39,44}

For each neuron, the longest basilar dendrite, including all branches, was reconstructed in 3 dimensions with a tracing system (Neuron Tracing System; Eutectics Electronics Inc, Raleigh, NC) and a $\times 100$ oil immersion objective (**Figure 1**). Only those portions of the dendritic tree within the same section as the cell soma were reconstructed. Because apical dendrites are frequently truncated during sectioning, these dendrites were not examined. Each dendritic branch was recorded as having either a natural end (gradual tapering of dendritic thickness with

an end swelling, spine, or spine cluster) or an artificial end (cut dendrite).³⁹ For each basilar dendrite and its branches, the mean diameter and total length, the location and number of spines, the total number of dendritic segments (the portion of a dendrite located between either the soma or a dendritic bifurcation and either another bifurcation or the dendrite end), and the maximum branch order (highest numbered dendritic segment) of the dendrites were determined. The cross-sectional area of each cell body was determined by tracing its outline. All neurons were reconstructed by the same investigator (L.A.G.) without knowledge of the subject number or diagnostic group.

STATISTICAL ANALYSES

For each parameter measured, the results were analyzed using a multivariate analysis of covariance model with the independent variables of diagnostic group, sex, age, race, PMI, and tissue fixation time. The multiple observations per subject (15 neurons) for a particular parameter were treated as a multivariate observation. Because the measured parameters within a given subject were possibly correlated and were also exchangeable, they were modeled as repeated measures with a compound, symmetric covariance structure. To preserve degrees of freedom, the interactions with diagnostic group were only examined if the effect of a particular independent variable was significant ($P < .05$). For any neuron parameter in which the multivariate analysis of covariance test yielded a significant diagnostic group effect at the .05 level, post hoc simultaneous pairwise comparisons using the Bonferroni procedure at the .05 level were conducted to determine which of the groups' means differed significantly. Simultaneous 95% confidence intervals were also obtained for each pairwise comparison of diagnostic groups. Finally, paired *t* tests were used to compare spine density across layers within subject groups.

ence significant at $P = .004$ by post hoc comparisons. Furthermore, although spine density did not differ ($t = 0.52$, $P = .61$) between superficial and deep layer 3 pyramidal neurons in the normal controls (Table 3), spine density was significantly ($t = 3.65$, $P = .003$) decreased by 11% in deep layer 3 relative to superficial layer 3 in the subjects with schizophrenia. These comparisons confirm a laminar specificity to the spine density differences between schizophrenic and normal control subjects.

Compared with the psychiatric subjects, the mean spine density on deep layer 3 pyramidal neurons in the schizophrenic subjects was decreased by 16%. Although this difference did not achieve statistical significance ($P = .08$), the 95% confidence interval (-35.8% to 4.4%) was suggestive of a reduction in spine density in the schizophrenic subjects compared with the psychiatric subjects. In contrast, the mean spine density clearly did not differ ($P = .81$) between the psychiatric and control subjects.

In contrast to area 46, the spine density on layer 3 pyramidal neurons in area 17 (Figure 3, C), primary visual cortex, was decreased in the schizophrenic (13%) and psychiatric (11%) subjects relative to the normal con-

trols (**Table 4**). However, these differences did not achieve statistical significance ($F_{2,34} = 2.70$, $P = .08$).

OTHER PARAMETERS OF LAYER 3 PYRAMIDAL NEURONS

In superficial layer 3 of area 46, only somal size significantly ($F_{2,37} = 3.84$, $P = .03$) differed among the 3 groups, and post hoc comparisons revealed that this difference was due to a smaller somal size in the psychiatric subjects compared with the normal controls (Table 3). In deep layer 3 of area 46, only total dendritic length (TDL) differed significantly ($F_{2,37} = 4.17$, $P = .02$) among the 3 groups. Post hoc comparisons revealed that the normal control group had a significantly ($P < .05$) greater TDL than the schizophrenic and psychiatric groups, which did not differ from each other. Interestingly, an analysis of covariance for spine density, controlling for TDL, in the schizophrenic and control groups revealed that the group difference in spine density on deep layer 3 pyramidal neurons was more highly significant ($F_{1,27} = 15.2$, $P < .001$) than that indicated by the initial analysis. In layer 3 of the primary visual cortex, TDL ($F_{2,34} = 4.11$, $P = .03$),

Table 1. Characteristics of Control, Schizophrenic, and Psychiatric Subjects^a

Control Subjects				Schizophrenic Subjects					
Subject No./ Sex/Age, y	Race	PMI, h	Cause of Death	Subject No./ Sex/Age, y	Race	Diagnosis	PMI, h	Duration of Illness, y	Cause of Death
370/M/25 ^b	W	5.3	Trauma	398/F/41	W	Schizoaffective disorder	10.3	20	Pulmonary embolism
390/F/72	W	11.0	ASCVD	410/M/30	B	Schizophrenia, chronic paranoia ^d	21.0	NA	Sickle-cell crisis
471/M/44	W	8.5	Hypertrophic cardiomyopathy	428/F/67	W	Schizoaffective disorder ^e	9.0	30	COPD
510/M/63	W	12.4	GI tract bleeding	450/M/48	B	Schizophrenia, chronic undifferentiated ^{d,g,h}	22.0	24	Suicide by jumping
516/M/20	B	14.0	Homicide by gun shot	466/M/48	B	Schizophrenia, chronic undifferentiated	19.0	NA	ASCVD
545/M/65	B	13.1	Pancreatic cancer	517/F/48	W	Schizophrenia, chronic disorganized ^d	3.7	20	Intracerebral hemorrhage
546/F/37	W	23.5	ASCVD	533/M/40	W	Schizophrenia, chronic undifferentiated	29.1	15	Accidental asphyxiation
551/M/61	W	16.4	Cardiac tamponade	537/F/37	W	Schizoaffective disorder ^d	14.5	8	Suicide by hanging
557/M/47	W	15.9	ASCVD	559/F/61	W	Schizoaffective disorder ^f	16.8	20	ASCVD
567/F/46	W	15.0	Mitral valve prolapse	566/M/63	W	Schizophrenia, chronic undifferentiated ^g	18.3	20	ASCVD
568/F/60	W	9.5	ASCVD	581/M/46	W	Schizophrenia, chronic paranoia ^{c,l}	28.1	30	Accidental combined drug overdose
575/F/55	B	11.3	ASCVD	587/F/38	B	Schizophrenia, chronic undifferentiated ^e	17.8	20	Myocardial hypertrophy
592/M/41	B	22.1	ASCVD	597/F/46	W	Schizoaffective disorder	10.1	25	Pneumonia
609/F/77	W	10.7	ASCVD	617/F/44	B	Schizophrenia, chronic undifferentiated	3.3	21	Amitriptyline hydrochloride toxicity
643/M/50	W	24.0	ASCVD	622/M/58	W	Schizophrenia, chronic undifferentiated ^d	16.0	16	Right MCA infarction

^aPMI indicates postmortem interval; NA, data not available; ASCVD, atherosclerotic coronary vascular disease; COPD, chronic obstructive pulmonary disease; GI, gastrointestinal; and MCA, middle cerebral artery.

^bAlcohol abuse, current at the time of death.

^cOther substance abuse, current at the time of death.

^dSubjects not taking antipsychotic medications at the time of death.

^eAlcohol abuse, in remission at the time of death.

^fAlcohol dependence, current at the time of death.

^gAlcohol dependence, in remission at the time of death.

^hOther substance dependence, in remission at the time of death.

ⁱOther substance dependence, current at the time of death.

^jSubjects treated with antipsychotic medications.

^kOther substance abuse, in remission at the time of death.

number of branch segments ($F_{2,34} = 4.41, P = .02$), and maximum branch order ($F_{2,34} = 4.27, P = .02$) differed significantly among the diagnostic groups (Table 4). For each measure, the main effect was due to significantly lower values in the psychiatric subjects compared with the control and schizophrenic subjects.

EFFECT OF ANTIPSYCHOTIC MEDICATIONS ON SPINE DENSITY

To determine whether treatment with antipsychotic medications might account for the decreased spine density on DLPFC deep layer 3 pyramidal neurons in the schizophrenic subjects, we conducted a separate analysis of the 9 psychiatric subjects who had been treated with these medications (Table 1). The mean (\pm SD) spine density (measured as number of spines per micrometer) on deep

layer 3 pyramidal neurons in these subjects (0.30 ± 0.07) did not significantly differ from that of either the entire group of normal controls ($0.33 \pm 0.08, F_{1,18} = 0.47, P = .50$) or a subset of 9 normal controls ($0.31 \pm 0.05, F_{1,12} = 0.06, P = .81$) who, as a group, did not differ from the antipsychotic-treated psychiatric subjects in sex, age, or PMI.

COMMENT

These findings demonstrate that the density of basilar dendritic spines on deep layer 3 pyramidal neurons is significantly decreased in DLPFC area 46 of subjects with schizophrenia. This decrease does not appear to be a general correlate of having a psychiatric illness or a consequence of treatment with antipsychotic medications, suggesting that the decrease in spine density may be specific to the pathophysiological characteristics of schizophre-

Psychiatric Subjects

Subject No./ Sex/Age, y	Race	Diagnosis	PMI, h	Duration of Illness, y	Cause of Death
453/M/37	W	Major depression ^{b,c}	11.0	NA	Suicide by gunshot
475/M/17	W	Major depression	23.0	1	Suicide by gunshot
505/M/57	W	Major depression ^f	12.8	18	Suicide by gunshot
513/M/24	W	Major depression with psychotic features ⁱ	13.1	8	Suicide by hanging
532/M/76	W	Mood disorder due to general medical condition with psychotic features ⁱ	13.9	0.3	Suicide by jumping
560/M/46	B	Alcohol-induced psychotic disorder ^{f,i}	10.6	2	GI bleeding
564/F/56	W	Major depression with psychotic features	16.6	1	Suicide by hanging
565/F/62	W	Major depression ^{b,h}	12.4	14	Suicide by gunshot
580/F/58	W	Major depression with psychotic features ⁱ	11.3	34	ASCVD
613/M/59	W	Major depression with psychotic features ^{a,i}	15.6	9	Suicide by gunshot
631/M/45	W	Major depression with psychotic features ^{b,i}	12.1	23	Suicide by thioridazine overdose
632/F/77	W	Delusional disorder ^f	17.4	36	ASCVD
637/M/34	W	Alcohol-induced mood disorder with psychotic features ^{f,i,k}	24.7	12	Suicide by chlorpromazine overdose
642/F/40	W	Bipolar disorder ^{f,i}	24.1	14	Acute alcohol intoxication
693/F/42	W	Major depression with psychotic features ^{i,j}	12.6	24	Suicide by propoxyphene hydrochloride overdose

nia. Because dendritic spine density directly reflects the number of excitatory inputs to pyramidal neurons,^{35,36} these findings, in concert with those of a pilot study⁴⁵ that also reported decreased spine density on prefrontal layer 3 pyramidal neurons, support the hypothesis that schizophrenia is associated with diminished synaptic connectivity of the DLPFC.

In superficial layer 3 of area 46 and in layer 3 of area 17, pyramidal cells exhibited a trend toward decreased spine density in the schizophrenic subjects compared with the normal controls. Evidence suggestive of decreased cortical neuropil has been reported in both of these cortical regions.¹⁰ However, in area 17, the psychiatric subjects also showed a trend toward decreased spine density on layer 3 pyramidal neurons. In addition, other measures of dendritic morphologic characteristics (TDL, number of branch segments, and maximum branch order) appeared to be altered in area 17 of the psychiatric sub-

jects, suggesting that a history of depression or death by suicide may be associated with altered neuronal morphologic characteristics in the primary visual cortex.

Interpretation of the pathophysiological significance of reduced spine density in the DLPFC requires a consideration of the potential influence of other factors. First, only a small percentage of neurons are labeled with the Golgi technique.⁴⁶ However, because the impregnation process is random, the cells reconstructed in this study are likely to be representative of the neuronal populations of interest. This interpretation is supported by our finding of a 9% to 12% decrease in mean somal size of layer 3 pyramidal neurons in the DLPFC of the schizophrenic subjects. Although these differences were not significant, perhaps because of sample size, their magnitude is consistent with that of other reports^{47,48} that measured somal size in much larger samples of Nissl-stained neurons. Second, because the reaction product

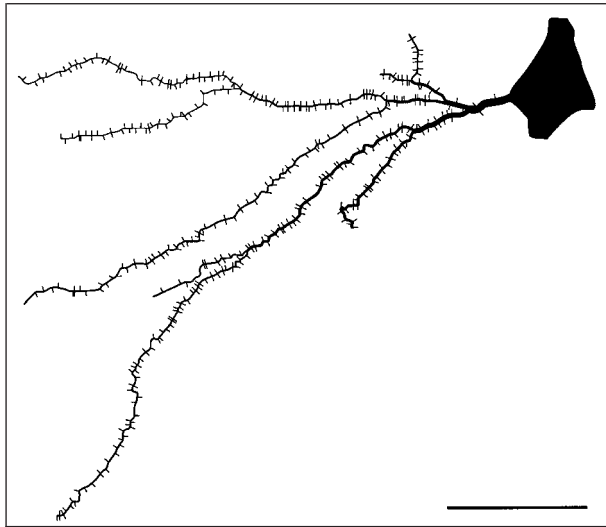


Figure 1. Reconstruction of a basilar dendrite from a layer 3 pyramidal neuron in control subject 516. Fourth-order dendritic branches are present. For each neuron, calculation of dendritic spine density included the total length of the dendrite shown. The bar indicates 50 μ m.

Table 2. Summary of Subject Characteristics*

Characteristic	Subject Group			P†
	Control	Schizophrenic	Psychiatric	
Male-female ratio	9:6	7:8	9:6	.71
White-black ratio	11:4	10:5	14:1	.20
Age, y	50.9 \pm 16.2	47.7 \pm 10.5	48.7 \pm 17.2	.84
PMI, h	14.2 \pm 5.5	16.1 \pm 7.7	15.4 \pm 4.8	.73
Fixation time, mo	12.5 \pm 10.1	10.7 \pm 9.4	8.2 \pm 6.6	.43
Alcohol disorder‡	1 (7)	7 (47)	8 (53)	.59
Other substance disorder	0 (0)	2 (13)	5 (33)	.17
Age at onset, y§	NA	28.6 \pm 9.4	36.7 \pm 18.3	.23
Duration of illness, y§	NA	20.7 \pm 5.9	14.0 \pm 11.8	.09
Out-of-hospital deaths	13 (87)	11 (73)	14 (93)	.19
Suicide	0 (0)	2 (13)	11 (73)	.005

*Data are given as number (percentage) of subjects or mean \pm SD. PMI indicates postmortem interval; NA, data not applicable.

†Analyses were between all 3 groups for the following variables: sex, race, age, PMI, fixation time, and out-of-hospital deaths. Analyses were conducted only between the schizophrenic and psychiatric groups for the following variables: alcohol disorder, other substance disorder, age at onset, duration of illness, and suicide.

‡After the study was initiated, 1 control subject (subject 370) was determined to meet the criteria for alcohol abuse, current at the time of death.

§This information could not be reliably determined for 2 schizophrenic and 2 psychiatric subjects.

of the Golgi impregnation procedure is opaque, some spines are hidden behind the dendritic shaft and are not counted. Consequently, the spine densities reported in this study are relative and not absolute. However, previous studies⁴⁹ have demonstrated that relative spine counts accurately reflect absolute numbers if comparisons are made between dendrites with similar shaft diameters, and, as shown in Tables 3 and 4, mean dendritic diameter did not differ across our subject groups. Third, the schizophrenic and psychiatric subjects available for this study were somewhat diagnostically heterogeneous. However, the mean (\pm SD) spine density in DLPFC deep layer 3 of the subjects with “pure” schizophrenia

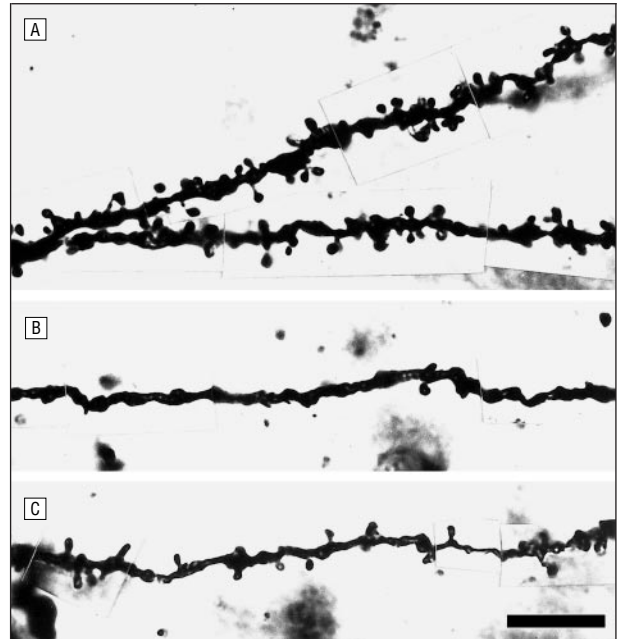


Figure 2. Brightfield photomicrographs illustrating Golgi-impregnated basilar dendrites and spines on dorsolateral prefrontal cortex layer 3 pyramidal neurons from normal control subject 390 (A) and 2 subjects with schizophrenia (subjects 410 [B] and 466 [C]). The calibration bar equals 10 μ m.

(0.25 \pm 0.06; n = 10) was 23% lower than that of the normal controls (0.32 \pm 0.07; n = 14) and of the psychiatric subjects with major depression (0.33 \pm 0.04; n = 10).

All of the schizophrenic subjects in this study had a history of treatment with antipsychotic agents. Studies addressing the effect of haloperidol on spine density in the rat prefrontal cortex have been inconclusive, with spine density reported to be decreased following high-dose, short-term treatment⁵⁰ but unchanged following long-term treatment at levels more consistent with clinical practice.⁵¹ However, 3 lines of evidence suggest that antipsychotic medications do not account for the decreased dendritic spine density observed in the present study. First, the decrease in spine density exhibited regional and laminar specificity, an effect that is not readily explained by systemically administered agents. Second, the 4 schizophrenic subjects (subjects 410, 450, 537, and 622) who were not taking medications (for an average of 5.4 months) at the time of death actually had a lower spine density (0.24 \pm 0.08) in deep layer 3 of area 46 than did the 11 subjects who were taking medications (0.26 \pm 0.06). Finally, the 9 psychiatric subjects who had been treated with antipsychotic medications did not differ in spine density from normal controls. However, the lifetime exposure to antipsychotic medications is likely to have been lower in these psychiatric subjects than in the schizophrenic subjects.

Although decreased spine density represents a morphologic abnormality in the DLPFC of schizophrenic subjects, dendritic spines are relatively plastic structures. For example, spine number has been reported to change rapidly in certain brain regions of experimental animals under various conditions.⁵²⁻⁵⁷ Although we cannot completely exclude the influence of such factors, their impact may have been minimized by the study design. For ex-

Table 3. Dendritic Parameters From Layer 3 Pyramidal Neurons in the DLPFC (Area 46)*

Dendritic Parameter	Layer 3					
	Superficial			Deep		
	Control Subjects	Schizophrenic Subjects	Psychiatric Subjects	Control Subjects	Schizophrenic Subjects	Psychiatric Subjects
Spine density, spines/ $\mu\text{m}\dagger$	0.34 \pm 0.09	0.29 \pm 0.10	0.33 \pm 0.11	0.33 \pm 0.10 ^a	0.26 \pm 0.10 ^b	0.31 \pm 0.09 ^{a,b}
Somal area, $\mu\text{m}^2\dagger$	355.2 \pm 143.5 ^a	312.1 \pm 127.7 ^{a,b}	300.4 \pm 111.3 ^b	350.9 \pm 121.8	319 \pm 126.5	308.9 \pm 106.4
Total dendritic length, $\mu\text{m}\S$	656.1 \pm 308.9	566.8 \pm 342.7	547.9 \pm 308.5	707.7 \pm 352.6 ^a	576.6 \pm 319.8 ^b	557.4 \pm 303.6 ^b
Dendritic diameter, μm	0.44 \pm 0.09	0.44 \pm 0.1	0.42 \pm 0.08	0.42 \pm 0.08	0.47 \pm 0.13	0.44 \pm 0.08
No. of branch segments	10.8 \pm 4.2	10.4 \pm 4.8	10.6 \pm 4.7	11.5 \pm 4.8	10.4 \pm 4.6	11.0 \pm 4.5
Maximum branch order	4.4 \pm 1.0	4.3 \pm 1.1	4.3 \pm 1.1	4.6 \pm 1.1	4.3 \pm 1.2	4.4 \pm 1.1
No. of natural ends	3.3 \pm 1.9	3.3 \pm 2.0	3.1 \pm 1.8	3.2 \pm 1.9	3.1 \pm 1.9	3.1 \pm 1.9
No. of artificial ends	2.6 \pm 1.9	2.4 \pm 1.7	2.7 \pm 2.0	3.0 \pm 2.0	2.6 \pm 2.0	2.9 \pm 2.1

*Data are given as the mean \pm SD. $n = 15$ for all subjects. DLPFC indicates dorsolateral prefrontal cortex.

\dagger Group differences are significant in deep layer 3 only ($F_{2,37} = 6.01$, $P = .006$). Within this row, values not sharing the same superscript letter are significantly different ($P < .05$) by pairwise post hoc analyses.

\ddagger Group differences are significant in superficial layer 3 only ($F_{2,37} = 3.84$, $P = .03$). Within this row, values not sharing the same superscript letter are significantly different ($P < .05$) by pairwise post hoc analyses.

\S Group differences are significant in deep layer 3 only ($F_{2,37} = 4.17$, $P = .02$). Within this row, values not sharing the same superscript letter are significantly different ($P < .05$) by pairwise post hoc analyses.

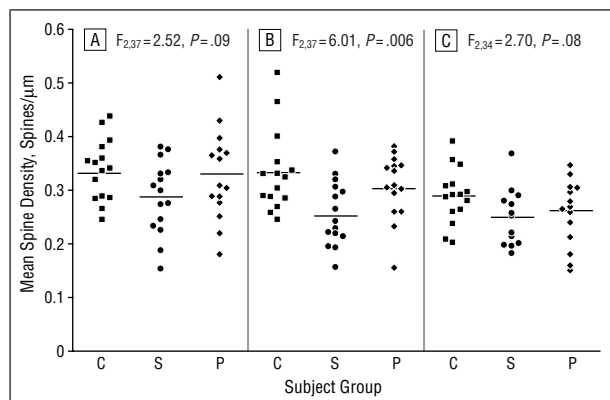


Figure 3. Scatterplots illustrating mean spine densities for 15 pyramidal neurons per subject in the superficial (A) and deep (B) portions of layer 3 in dorsolateral prefrontal cortex area 46, and in area 17, primary visual cortex (C). Horizontal lines indicate group means; C, control subjects; S, schizophrenic subjects; and P, psychiatric subjects.

ample, the comparison with subjects with mood or other psychotic disorders provides some assessment of the influence of environmental factors, such as hospitalizations, medications, and limitations in social and occupational activities, associated with being severely ill with a psychiatric disorder. In addition, spine density in deep layer 3 of area 46 was not associated with the duration of illness in either the schizophrenic ($r = -0.358$, $P = .26$) or psychiatric ($r = -0.009$, $P = .98$) subjects. However, the plasticity of dendritic spines suggests that the findings of this study, like many other observations in postmortem studies (alterations in gene expression or neurotransmitter receptor number), may not reflect a fixed lesion in the DLPFC of schizophrenic subjects.

Because the presynaptic and postsynaptic elements of axospinous synapses change in parallel,^{35,54,58-60} the decreased spine density in schizophrenic subjects is likely to reflect a diminished number of excitatory synaptic inputs to DLPFC layer 3 pyramidal neurons. This interpretation is consistent with previous reports^{10,11,23-25} of de-

Table 4. Dendritic Parameters From Layer 3 Pyramidal Neurons in the Primary Visual Cortex (Area 17)*

Dendritic Parameter	Control Subjects (n = 15)	Schizophrenic Subjects (n = 13)	Psychiatric Subjects (n = 14)
Spine density, spines/ μm	0.29 \pm 0.08	0.25 \pm 0.09	0.26 \pm 0.09
Somal area, μm^2	190.9 \pm 76.9	181.5 \pm 70.0	174.2 \pm 65.1
Total dendritic length, $\mu\text{m}\dagger$	449.3 \pm 227.9 ^a	464.5 \pm 325.0 ^a	351.6 \pm 178.5 ^b
Dendritic diameter, μm	0.41 \pm 0.11	0.43 \pm 0.10	0.40 \pm 0.10
Number of branch segments \ddagger	9.3 \pm 3.8 ^a	9.1 \pm 4.0 ^a	8.2 \pm 3.2 ^b
Maximum branch order \S	4.1 \pm 1.0 ^a	4.0 \pm 1.1 ^{a,b}	3.8 \pm 0.9 ^b
No. of natural ends	3.1 \pm 1.7	3.0 \pm 1.9	2.7 \pm 1.6
No. of artificial ends	2.0 \pm 1.6	2.1 \pm 1.9	1.9 \pm 1.5

*Data are given as the mean \pm SD.

\dagger Group differences are significant ($F_{2,34} = 4.11$, $P = .03$). Within this row, values not sharing the same superscript letter are significantly different ($P < .05$) by pairwise post hoc analyses.

\ddagger Group differences are significant ($F_{2,34} = 4.41$, $P = .02$). Within this row, values not sharing the same superscript letter are significantly different ($P < .05$) by pairwise post hoc analyses.

\S Group differences are significant ($F_{2,34} = 4.27$, $P = .02$). Within this row, values not sharing the same superscript letter are significantly different ($P < .05$) by pairwise post hoc analyses.

creased synaptophysin protein and neuropil measures in the DLPFC of schizophrenic subjects. Interestingly, dendritic spine density on layer 3 pyramidal neurons undergoes a substantial decline during adolescence in primates.³² In addition, the density of asymmetric (presumably excitatory) synapses changes in a similar manner in the monkey and human DLPFC.^{33,34} These late developmental refinements in the excitatory circuitry of the DLPFC coincide with the age when the clinical manifestations of schizophrenia frequently first appear, suggesting that they may contribute to the pathophysiological characteristics of this disorder.⁶¹ However, we cannot

determine from the present study whether the presynaptic terminals to DLPFC layer 3 pyramidal neurons never developed, were extensively pruned during adolescence, or were resorbed later in life.

The functional significance of a decrease in excitatory inputs depends on which population(s) of axon terminals is affected. Several lines of evidence suggest that the affected inputs may be from the mediodorsal thalamic nucleus. First, this nucleus has been reported to have fewer neurons in schizophrenic subjects.²⁹⁻³¹ Second, spine density was preferentially decreased on pyramidal neurons in deep layer 3 of the DLPFC. The basilar dendrites of these neurons typically extend through deep layer 3 and layer 4, the termination zone of afferents from the mediodorsal thalamic nucleus,²⁷ and dendritic spines appear to be the principal synaptic targets of thalamic projections.^{28,62} Finally, decreased expression of the messenger RNA for GAD₆₇, the synthesizing enzyme for γ -aminobutyric acid, in the DLPFC of schizophrenic subjects^{62,63} has been suggested to represent a compensatory response to diminished excitatory thalamic drive,⁶⁴ since decreased activity in thalamic inputs to sensory cortices produces a down-regulation of GAD₆₇ expression.^{65,66}

However, the observations of this study may not be fully explained by a reduction in thalamic inputs to the DLPFC. For example, thalamocortical afferents appear to compose a small proportion (<10%) of the total excitatory inputs to the targeted cortical neurons in the cat visual cortex.⁶⁷ If these findings can be extrapolated to the human DLPFC, then even a complete loss of thalamocortical afferents would not be sufficient to account for the observed 16% to 23% decrease in basilar dendritic spine density on deep layer 3 pyramidal cells in the schizophrenic subjects. Two other major sources of excitatory inputs to deep and superficial layer 3 DLPFC pyramidal neurons are intrinsic axon collaterals from other pyramidal neurons^{68,69} and associational or callosal projections from other cortical regions.⁶⁹⁻⁷¹ Thus, given the trend for spine density to also be decreased on superficial layer 3 pyramidal cells, it may be that abnormalities in thalamocortical afferents to deep layer 3 have an additive effect to a disturbance in cortical axon terminals that are distributed across layer 3. However, our findings do not reveal the direction of the pathophysiological changes. For example, it is possible that the inputs to DLPFC layer 3 pyramidal cells are reduced not because of a more primary disturbance in the source of the inputs but because an abnormality intrinsic to these pyramidal cells renders them unable to support a normal complement of excitatory inputs.

In summary, our findings provide evidence for a decrease in excitatory inputs to DLPFC layer 3 pyramidal cells that may be most marked for pyramidal cells located in the thalamic recipient zone. Given the role of thalamic excitatory inputs in the mediation of working memory,^{72,73} these findings may contribute to the pathophysiological basis for the disturbance of these cognitive abilities in subjects with schizophrenia.

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